

# LABORATORY NOTEBOOK

MBX-1212

Notebook No.: 6

Assigned to: Lara Madison

Date: \_\_\_\_\_

Use Nalge Cat. No.

6301-1000  
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Design primers for sequencing AI/BI Fusions

1296..2036 ="phbB"

20  
1296  
740

FB.1

FB.2

FB.3

atgac tcagcgcatt gcgtatgtga  
ccggcgcgc gggtggtatc ggaaccgcca ttgcccagcg gctggccaag ggtggtttc  
gtgtggtggc cgggtgcggc cccaactcgc cgcgcgcga aaagtggctg gacgcagcaga  
aggccctggg cttcgatttc attgectcgg aaggcaatgt ggctgactgg gactcgacca  
agaccgcatt cgacaaggte aagtcggagg tcggcgaggt tgaatgtctg atcaacaacg  
ccggtatcac ccgcgacgtg gtgttcgca agatgaccg cgcgcactgg gatcggtga  
tcgacaccaa cctgactctg ctgttcaacg tcaccaagca ggtgatcgac ggcatggccg  
accgtggctg gggccgcacg gtaacatct cgtcggtgaa cgggcagaa ggcagttcg  
gccagaccaa ctactccacc gccaaaggcg gectgcatgg cttcaccatg gactggcgc  
aggaagtggc gaccaaggcg gtgaccgtca acacggctc tccgggctat atcgccaccg  
acatggtcga ggcgatccgc caggacgtgc tcgacaagat gtcgcgacg atcccggtca  
agcgctggg cctgcggaa gagatcgct cgatctgcg ttggtgtcg tcggaggagt  
ccggtttctc gaccggcgcc gacttctgc tcaacggcg cctgcatatg ggctga

Table of Contents

40..1221 ="phbA"

FA.1

FA.2

FA.3

FA.4

a tgactgacgt tgtcatcgta  
tccgcgcgcc gcaccgcggt cggcaagttt ggcggtctgc tggccaagat ccggcaccg  
gaactgggtg ccgtggtcat caaggccgcg ctggagcgcg ccggcgtaaa gccggagcag  
gtgagcgaag tcactatggg ccaggtgctg accgcccgtt cgggccagaa ccccgacgc  
caggccgcga tcaaggccgg cctgcggcg atggtgccc ccatgacct caacaagggt  
tgcggtcgg gctgaaggc cgtgatgctg gccgccaacg cgatcatggc gggcgacgc  
gagatcgtg tggccgggg ccaggaaac atgagcg ccccgacgt gctgcgggc  
tcgcgcgatg gtttccgat gggcgatgcc aagctggtcg acaccatgat cgtcgacggc  
ctgtgggacg tgtacaacca gtaccacatg ggcacaccc cggagaacgt ggccaaggaa  
tacggcatca caccgcggc gcaggatgag ttcccgctcg gctcgcagaa caaggccgaa  
gccgcgcga aggcggcga gtttgacgaa gagatcgctc gtgtctgat cccgcagcg  
aaggcgac cgggtggcct caagaccgac gagttcgtgc gccaggcgcc cacgtggac  
agcatgtccg gectcaagcc cgccttcgac aaggccggca cggtgaccgc ggccaacgc  
tcgggcctga acgacggcg cgcgcgggtg gtggtgatgt cggcgccaa ggccaaggaa  
ctgggcctga ccccgctggc cagcatcaag agctatgcca acgcgggtgt cgatcccaag  
gtgatgggca tgggcccgtt gccggcctcc aagcgcgcc tgtcgcgcg cgagtggacc  
ccgcaagacc tggacctgat agagatcaac gaggcctttg ccgcgcaggc gctggcggtg  
eacccagcaga tgggctggga cactccaag gtcaatgtga acggcgcgcc catcgccatc  
ggccaccgca tcggcgctc gggctgctg atcctggtga cgctgtgca cgagatgaag  
cgccgtgacg cgaagaagg cctggcctcg ctgtgcatcg gccggcgcat gggcggtggc  
ctggcagtcg agcgcaata a

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Read and Understood By

Lisa L. Mullison10/2

Date

AB FUSION CHARACTERIZATION

noticed I had left the MBx240 pTrc AB fusion on the bench for approximately a week.

MBx240 pTrc AB # 10, 11, 12, 13

LB Amp clear / tan no granules

LB Amp white granules  
1% glucose

LB Amp white granules  
1% glucose  
0.1mM IPTG

eye

light microscopy

start LB glucose O/N culture from freezer stock.

50% x = 1% 100mM

2hr + 0.5hr

1/12-16-97 dilute cultures 1:100 into LB 1% glucose Amp

O/N

@ time dil

4 hrs post -

3hr after inoc.

induction

	O/N	@ time dil	4 hrs post -	induction
pTrc-AB 10	0.46335	4.63	0.7839	1.344
11	0.55087	5.51	0.7959	1.324
12	0.42090	4.21	0.7931	1.513
13	0.50844	5.84	0.7476	1.268

induced

⊕

⊖

5mM IPTG

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take 300ul

samples → 4°C

Continued on Page

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Read and Understood By

Lara Anderson

Signed

Date

Signed

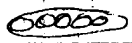
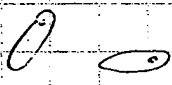

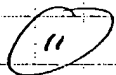
Date

Cont. from p. 88

LOOK @ CULTURES UNDER MICROSCOPE.  
DID NOT HAVE TIME WHEN I TOOK  
SAMPLES FOR WESTERN.

THEREFORE SAMPLES HAVE BEEN @ RT FROM 5 PM → 11:15 AM  
\* SAMPLES ARE IN LB 1% GLUCOSE \*

some cells had  
inclusion  
bodies  
w/out  
HB  
granules

	+ IPTG	-
PTCLAB #10	granules look like peas in a pod 	single granules @ poles 
#11		
#12	"	"
#13	"	"
	many cells w/ inclusion bodies	

Majority of cells in presence 5mM IPTG  
are 2-3x ~~more~~ normal length  
while the majority of cells without IPTG  
are 1x cell lengths.

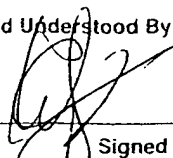
Of course there is always a small majority  
that are 5-10x cell length but only in  
IPTG samples

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Lana L. Mackson

Read and Understood By



Signed

Date

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Date

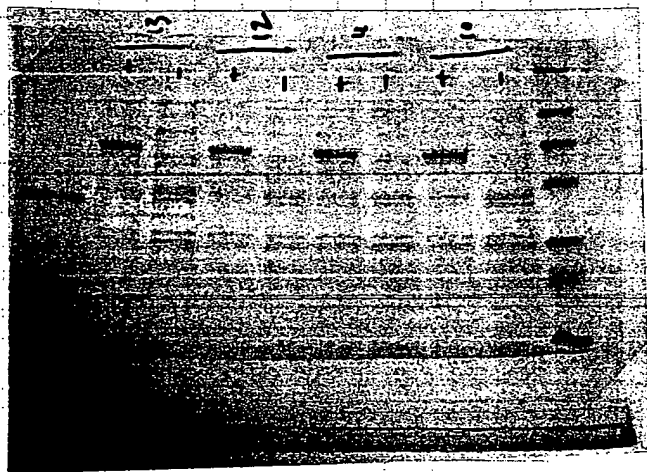
GEL &amp; WESTERN OF AB FUSIONS! p. 88

Lane	Sample	5 mM IPTG	OD <sub>600</sub>	ml for 200	due
(11) 1	MW				
(12) 2	pTrc AB #10	-	1.020	19.6	10
(13) 3		+	1.344	14.9	
(14) 4	pTrc AB #11	-	1.003	20.0	
(15) 5		+	1.324	15.1	
(16) 6	pTrc AB #12	-	0.9694	20.6	
(17) 7		+	1.513	13.2	
(18) 8	pTrc AB #13	-	0.9897	20.2	
(19) 9		+	1.268	15.7	
(20) 10					

$$\frac{200 \text{ (19 ml)}}{\text{ml}} = 1.020 \times$$

19.0 = 27.15 ml bottles  
 10 = 2 x 5 ml brown cap  
 2.12 = 10 ml dye

2 gels → 1 coomassie  
 1 western



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Read and Understood By

Lucia L. Madson

Signed

Date

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